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**Original Article**

# *Testing the Effect of Grape Seed Extract (Vitis Vinifera L) on the Healing Process of Cut Wounds in Wistar White Rats*

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***ABSTRACT***

Daily actions cause wounds. Normal and abnormal wounds generate inflammation. Grape (*Vitis vinifera l*) possesses antioxidant and wound-healing properties. The research aims to test grape seed extract's ability to heal cuts in white Wistar rats. The study is conducted solely on white Wistar rats. The type of research used was lab-experimental quantitative research with SPSS data. The study sample consisted of white Wistar rats (*Rattus norvegicus*). In this trial, grape seed extract was given at 50, 100, 150, and 200 mg/kg BW. Animals were sliced at 2 cm length with a depth of ± 2 mm to the dermis layer and treated according to their group. Grape seed extract cream healed mice-cut wounds. On the 14th day, group P0 had 72.3% wound healing, group P1 84.3%, group P2 94%, group P3 93.9%, and group P4 100%. Thus, group P4 healed faster than P0, P1, P2, and P3. Grape seed extract cream accelerates skin regeneration and stimulates fibroblasts more than base cream, according to wound healing percentages. Grape seed extract at 200 mg/kgBW accelerated wound healing more than other treatments. The entire treatment group demonstrated considerable normalcy with a 0.200 > 0.05 score. Four homogenous test groups had a significance value of 0.200 > 0.05. The one-way ANOVA test yielded a significance value of 0.000 < 0.05. The research concluded that grape seed extract contains saponins, alkaloids, steroids, and flavonoids that may serve as anti-inflammatory agents and antioxidants to inhibit free radicals and speed wound healing in rats.

***Keywords:*** *Grape Seed Extract, Wounds Healing, Antioxidants*

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## INTRODUCTION

The epidermis, dermis, and hypodermis cover the body and have varied roles and structures[1]. Due to its external nature, skin is especially susceptible to environmental stress. Damage or disturbance can undermine skin integrity [2]. Skin damage can cause wounds.

Wounds can result from physical contact, medicinal procedures, or physiological changes. Changes can disrupt anatomy's structure and function. Bites falls, and sharp item slashes can hurt animals [3].

Daily activities cause many injuries. Animal bites, temperature changes, chemicals, explosions, electric shocks, or sharp or blunt objects can cause this sickness. Open and closed wounds are categorized by their cause. Open wounds include cuts, abrasions, shallow wounds, knife wounds, penetration wounds, and lacerations [4]. Wounds heal automatically after damage.

The natural, dynamic, and interactive wound healing process incorporates soluble mediators, extracellular matrix, blood cells, and parenchymal cells. Wound healing has three stages: inflammatory, proliferative, and remodeling. Remodeling is crucial since it determines healing tissue strength and appearance [5].

Inflammation occurs during normal and pathological wound healing. The body's innate immune system activates quickly after injury, causing localized inflammation. In response to

host tissue responses, inflammatory cells are drawn from circulation. Oxidative stress results from increased ROS production in chronic inflammation. ROS regulates proper tissue repair and healing through many methods [6]. However, ROS levels in inflammatory tissue can surpass 500 µM, far more significant than normal tissue (1-15 µM) under certain clinical situations. Since skin tissue is prone to oxidative stress, excessive ROS generation may cause protein malfunction, aberrant cellular connections, DNA/RNA damage, and cell apoptosis. Antioxidants suppress molecular oxidation and restore healthy ROS levels to overcome these restrictions [7,8].

C-reactive protein (CRP) levels indicate inflammation [9]. The most sensitive sign of inflammation is CRP. Blood proteins include CRP. Liver CRP production rises after infection, injury, or inflammation. Acute phase proteins like CRP rise in response to inflammation. CRP levels rise during active psoriasis and fall following treatment. CRP reduces neutrophil activation and chemostatic response [9, 10, 11].

Normal wound healing requires minimal ROS and oxidative stress [12]. ROS also mediates cell signaling and inflammation in wound repair. ROS production is physiological yet hazardous in excess [4]. A wound dressing with antioxidants to fight free radicals is needed to expedite wound healing. Wound dressings protect and hasten healing. Wound contraction is constantly present, and the right amount of contraction speeds up wound healing [13,14,15, 16.]

Plants can produce many phytochemicals due to their inherent genetic makeup [17,18]. These compounds aid in regular physiological processes and protect harmful microorganisms and herbivores [19,20]. The grape plant is one of several therapeutic plants with antioxidant and wound-healing capabilities [21,22]. You can eat grapes (*Vitis vinifera L.*) or use them to make other foods and drinks, like raisins, grape juice, and fruit salad. Phytochemicals, dietary fiber, minerals, vitamins, carbs, and protein are all found in grapes [19,23,24]. This study aimed to examine and evaluate the efficacy of grape seed extract (*Vitis vinifera L*) in hastening the recovery time of white Wistar rats' (*Rattus norvegicus*) cuts.

## METHOD

This research uses a type of experimental quantitative research, namely a true experiment or laboratory experimental design that employs a post-test group design [25]. The histopathology laboratory testing and acclamation of test animals are conducted at the Animal House, Faculty of Mathematics, Natural Sciences, University of North Sumatra, from 02 August to 30 December 2023. The research sample was 25 male Wistar rats (Rattus norvegicus). The Federer formula calculated the sample size: (n-1) (t-1) > 15 [26]. According to calculations, each treatment group had at least six animals. The minimal sample size is 25 mice. A total of 25 animals were sampled from 5 treatment groups of 5 animals each. Since fewer than fifty participants were in this study, the researchers resorted to the Shapiro-Wilk statistical test. With a significance level of 0.05, statistical analysis is revealed by the Shapiro-Wilk test's significance value [27].

The variables in this study were the independent variable, namely the administration of grape seed extract (*Vitis vinifera L.*); the dependent variable, namely the healing of cuts and histopathological features; the precondition variables, namely cuts on the rats' backs and Biomarkers are CRP and SOD levels.

Method and Measuring Tools for Grape Seed Extract: Measure the dose with the help of a needle and administer it using a blunt probe. To heal rat wounds, use a caliper to examine blood samples taken from the orbital vein and histopathologically describe mouse skin tissue given an incision using a microscope with Olympus BX51® light microscope and Olympus DP20® microscope camera.

The tools used in this research are a macerator, analytical balance, glass stirring rod, glass funnel, vacuum rotary evaporator, cup, measuring cup, glass beaker, oven, water bath, autoclave, incubator, laminar air flow, test tube, petri dish tube rack, L rod, round tube needle, dropper pipette, micropipette, vortex, caliper, microscope, object glass, and hot plate. The test materials used in the research were grape seeds (Vitis vinifera L.), NaCl 0.9%, distilled water, ethanol 70%, spirits, and Mayer's reagent.

The research procedures carried out in this study began with the acclimation of test animals; Mice in lab cages were used for animal acclimatization. Plastic (30 cm x 20 cm x 10 cm) rat cages have tiny wire mesh. During the investigation, a daily change of 0.5–1 cm thick rice husks covered the cage bottom. The room temperature was 25–27 °C, the humidity was

35–50%, and the lighting was controlled to cycle 12 h light/12 h dark. Mice ate regular mouse pellets and drank distilled water freely.

Maceration with 70% ethanol produces grape seed extract. Grape seed powder is macerated with 1:10 powder: solvent for 24 hours, stirring every 6 hours. Remaceration was repeated by immersing the filter residue in an additional solvent until the macerate became clear, indicating that all chemicals were attracted. A rotary evaporator evaporated the filtrate to get a thick extract.

Phytochemical experiments were conducted to determine if grape seed extract included any chemicals that could speed up the healing process of wounds. The tannin content test is carried out by putting 1 gram of extract into a test tube, adding 10 mL of hot water, then boiling for 5 minutes, then adding 3-4 drops of FeCl3 to the filtrate. If the color is blue-green (green-black), it is positive for containing catechol tannins, whereas if it is blue-black, it means positive and contains pirogalo tannin.

The flavonoid content test requires 1 gram of sample extract to be put into a test tube, then concentrated HCl added, and then heated for 15 minutes in a water bath. If a red or yellow color is formed, it is positive for flavonoids (flavone, chalcone, and aurone). Two grams of sample extract are placed in a test tube, dripped with 5 mL of 2 N HCl, heated, cooled, and divided into three 1 mL test tubes for the Alkaloid Content Test. Reagents are added to each tube. If Mayer's reagent precipitates white or yellow, alkaloids are present. Wagner's reagent detects alkaloids if a brown precipitate appears. The Dragendrof reagent contains alkaloids and gives an orange residue.

Two grams of sample extract are placed in a test tube with 2 mL of ethyl acetate and agitated for the Steroid/Terpenoid Content Test. The ethyl acetate layer was dropped onto a drop plate to dry. After drying, two drops of acetic acid and one drop of concentrated sulfuric acid were added. Terpenoids are present if they become red or yellow. Steroids are present if they turn green. For the Saponin Content Test, place 1 gram of sample extract in a test tube, add 10 ml of boiling water, calm, and shake vigorously for 10 seconds. Saponin is present if the foam is 1-10 cm high in 10 minutes and does not dissolve after adding one drop of 2 N HCl.

Measure the absorbance of a 70 ppm vitamin C solution for the Antioxidant Activity Test in Determining the Maximum Wavelength. Combine 1 mL of this solution with 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide and incubate at 50 °C for 20 minutes. After incubation, 1 ml of TCA was added, centrifuged at 3000 rpm for 10 minutes, put into a 10 ml measuring flask, added 1 ml of distilled water and 0.5 ml of FeCl3, added enough oxalic acid to the limit mark, and measured absorption with a UV-Vis spectrophotometer calibrated from 400-800 to obtain the maximum wavelength.

After determining the maximum wavelength, the operating duration is tested to determine when the reaction is most stable and read the absorbance at 1 to 30 minutes.

Evaluation of Grape Seed Ethanol Extract Antioxidant Activity to get 1000 ppm, dissolve 25 mg grape seed extract in 25 ml ethanol p.a in a 25 ml volumetric flask. Add 20 µl, 40 µl, 60 µl, 80 µl, and 100 µl of stock solution to test tubes at concentrations of 2 ppm, four ppm, six ppm, eight ppm, and ten ppm, followed by 1 ml of phosphate buffer. 1 ml K3Fe (CN)6 1% and 0.2 N (pH 6.6). Incubate at 50°C for 20 minutes. Following incubation, one cc of 10% TCA solution was added and centrifuged for 10 minutes at 3000 rpm. After centrifuging, pipette it into a 10 ml measuring flask and add 0.5 ml of 0.1% FeCl3 and distilled water to the mark. Then, the most excellent wavelength absorption is calculated.

Test animals were acclimated before incision wounds were made on the backs of mice. Using a sterile scalpel with blade no. 4, ± 2 cm long incisions were made with a depth of 0.2 cm or up to the subcutaneous layer or hypodermis. The left index finger and thumb squeeze or stretch the skin.

In the control group, mice were only given 0.9% NaCl. Meanwhile, mice were given grape seed extract (Vitis vinifera L) in the treatment group at different doses. Treatment procedures for five groups for 14 days given rat pellets + distilled water, namely the control group (P-0): Only rat pellet feed + distilled water, Treatment Group I (P-1): Plus grape seed extract at a dose of 50 mg/kg BW, Treatment Group II (P-2): Plus grape seed extract at a dose of 100 mg/kg BW, Treatment Group III ( P-3): Plus grape seed extract at a dose of 150 mg/kg BW and Treatment Group IV (P-4): Plus grape seed extract at a dose of 200mg/kg BW.

Next, the wound observation process is carried out according to the wound's length and the length of time it takes to heal. The duration

of the observations was up to 14 days, corresponding to the average time it takes for a wound to heal to the proliferation phase, which typically occurs between 3 and 14 days after the wound originated. The mice were euthanized after 14 days by inhaling excess technical chloroform.

First and 15th-day blood samples were taken to test CRP and SOD. A 1 cc hematocrit capillary pipette was used to draw tail blood, and heparin was given as an anti-coagulant on the first and last days following acclimatization. Initial levels are taken on the first day. ELISA and 450 nm spectrophotometry examined SOD levels. The evaluation was done with the SOD kit (Brand Bioassay TL, EO168Ra). Mouse super oxidase dismutase ELISA kit was calculated using a standard curve equation. Expert health personnel at the University of North Sumatra laboratory examined secondary data for CRP. Mean CRP levels were classified as low (<0.1 mg/0.1), moderate (1.0 to 3.0 mg/L), and high (>3.0 mg/L) [28].

Anesthetized mice (0.1mg/200 ketaminexylazine) were used to prepare histopathological samples. Histopathological studies used mouse skin samples from healed lesions in a 10% buffered formalin solution. Healed skin samples were sectioned at 5 µm thickness and stained with H&E. The materials were maintained in a 40% PFA pot at room temperature and delivered to the University of North Sumatra Histopathology Laboratory for preparation. Histopathological observation with preparations at the wound edge during wound healing using an Olympus BX51® light microscope with 100x and 400x magnification and an Olympus DP20® microscope camera.

Re-capitalization revealed histopathological findings. Epithelial thickness scores: Score 1 (Reepithelialization of 0-25% of the wound part of normal), Score 2 (25-50%), Score 3 (50-75%), and Score 4 (>75%).

The Kolmogorov-Smirnov test was used to standardize the data in this investigation. Distribution is considered normal when P > 0.05. Apply Levene's test for homogeneity after data normalization. Homogeneity is shown by a P-value greater than 0.05 [27]. Following tests for homogeneity and normalcy, the SPSS analytical tool compares groups using the t-test.

## RESULTS

This study used 25 mice with 160-200-gram Wistar white rats. The Wistar strain was preferred for studies involving the body's metabolism because of the rats' comparatively high metabolic rate. Since estrogen is not present in male mice or in tiny amounts, and female mice undergo hormonal changes during estrus, nursing, and pregnancy, it was determined that male mice would be the best test subjects [29].

Accordingly, a normalcy test, applicable only when there are fewer than 30 observations, must be performed to ascertain whether the error term is approaching a normal distribution. Due to the small size of the research sample (less than 50), the Shapiro-Wilk statistical test was employed [27].

This study employs grape seed extract (Vitis vinifera L) at various dosages to speed up white Wistar rat back wound healing.

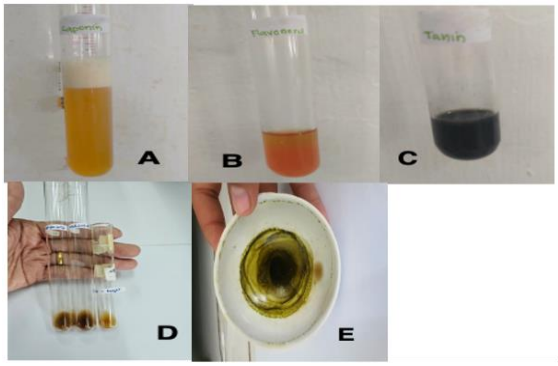
A cut wound is given to mice to start treatment. Shave the back area till it is clean (bald) to fit the required incision size (2x2cm). After shaving, the rats were deprived of consciousness with ketamine (80 ml/kg BW) and xylazine (5 ml/kg BW) to prevent pain and excessive movement, which wounded the rat's back. Four ± 2 cm incisions are performed with a 0.2 cm wound depth, possibly reaching the subcutaneous layer or hypodermis. The left index finger and thumb squeeze or stretch the skin.

After the wound was developed, the mice received four doses of grape seed extract (Vitis vinifera L). The P-0 control group received rat pellets and distilled water. Treatment Group I (P-1) received 50 mg/kg BW grape seed extract, treatment group II received 100 mg/kg BW, treatment group III received 150 mg/kg BW, and treatment group IV received 200 mg/kg BW. Observations were done for up to 14 days because the usual wound healing process takes between 3 and 14 days to reach the proliferation phase. They were inhaling excess technical chloroform that killed all mice after 14 days. From the first day of therapy to the 14th day, white Wistar rats (Rattus norvegicus) were measured for wound length to determine healing.

Figure 1-A shows the phytochemical

screening test findings for grape seed extract cream (Vitis vinifera L), a secondary metabolite chemical. With the Aquades reagent, 1 gram of grape seed extract is added to a test tube, and 10 ml of hot water is added, cooled, and shaken violently for 10 seconds. One drop of 2 N HCl creates a 1-10 cm foam in 10 minutes. It does not vanish, indicating saponin.

Figure 1-B. Put 1 gram of grape seed extract in a test tube, add strong HCl, and heat for 15 minutes in a water bath. Flavonoids are present if the test turns reddish-yellow. See Figure 1-C. A test tube with 1 gram of grape seed extract, 10 mL of hot water, and 5 minutes of boiling yielded a blue-green liquid with three to four droplets of FeCl3. This test proved positive for tannin.




Note : A: Saponin, B: Flavonoid, C: Tannin, D. Alkoloid, D: Steroid

**Figure 1. Phytochemical Test Results**

Figure 1-D shows 2 grams of Grape Seed Extract in a test tube, dripped with 5 mL of 2 N HCl, heated, cooled, and then divided into 3 1 mL test tubes. Reagents are added to each tube. When Mayer's reagent is introduced, a white or yellow precipitate appears. Wagner's reagent produced a brown residue. The Dragendrof reagent produced an orange precipitate, proving all these tests contained alkaloids. See Figure 1-E. Add 2 mL of ethyl acetate to 2 grams of grape seed extract in a test tube and shake. The ethyl acetate layer was dropped onto a drop plate to dry. After drying, two drops of acetic acid and one drop of sulfuric solid acid produced a green hue, indicating steroid presence.

Researchers monitored mouse wound healing daily and measured its length. Researchers calculated the average wound length every day from the first day the wound was formed to the 14th day to assess white mouse wound healing.

Wound lengths for each group of mice are compared on average in Table 1. The results show that the P0 group had the most extended wound at 0.554 cm, while the P4 group had the most minor wound at 0 cm. Each wound's length is expressed as a percentage of its pre-treatment length, with a value of 0.00%, so that we may compare the healing rate of cut wounds between treatments. Therefore, all research patients had the same proportion of wound healing before therapy.

**Table 1.** Mean Length And Percentage Of Wound Healing

| Days | Mean Wound Healing | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Group P0 | | Group P1 | | Group P2 | | Group P3 | | Group P4 | |
| | Cm | % | Cm | % | Cm | % | Cm | % | Cm | % |
| 1 | 2.000 | 0.0 | 2.000 | 0.0 | 2.000 | 0.0 | 2.000 | 0 | 2.000 | 0.0 |
| 2 | 1.952 | 2.4 | 1.922 | 3.9 | 1.876 | 6.2 | 1.888 | 5.6 | 1.888 | 5.6 |
| 3 | 1.904 | 4.8 | 1.844 | 7.8 | 1.746 | 12.7 | 1.678 | 10.65 | 1.678 | 16.1 |
| 4 | 1.874 | 6.3 | 1.784 | 10.8 | 1.634 | 18.3 | 1.512 | 21.4 | 1.512 | 24.4 |
| 5 | 1.756 | 12.2 | 1.642 | 17.9 | 1.438 | 28.1 | 1.298 | 35.6 | 1.218 | 39.1 |
| 6 | 1.666 | 16.7 | 1.46 | 27.0 | 1.254 | 37.3 | 1.148 | 44.1 | 1.048 | 47.6 |
| 7 | 1.572 | 21.4 | 1.252 | 37.4 | 1.082 | 45.9 | 0.986 | 52.5 | 0.900 | 55.0 |
| 8 | 1.470 | 26.5 | 1.170 | 42.0 | 0.918 | 54.1 | 0.900 | 57.6 | 0.748 | 62.6 |
| 9 | 1.266 | 36.7 | 0.918 | 54.1 | 0.720 | 64.0 | 0.782 | 64.2 | 0.616 | 69.2 |
| 10 | 1.182 | 40.9 | 0.730 | 63.5 | 0.608 | 69.6 | 0.588 | 66.3 | 0.474 | 76.3 |
| 11 | 0.964 | 51.8 | 0.632 | 67.9 | 0.522 | 73.9 | 0.476 | 71.3 | 0.276 | 86.2 |
| 12 | 0.832 | 58.4 | 0.514 | 74.3 | 0.366 | 81.7 | 0.322 | 85.0 | 0.100 | 95.0 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 13 | 0.732 | 64.0 | 0.420 | 79.0 | 0.204 | 89.8 | 0.244 | 87.5 | 0.000 | 100.0 |
| 14 | 0.554 | 72.3 | 0.314 | 84.3 | 0.120 | 94.0 | 0.122 | 93.9 | 0.000 | 100.0 |

**Note**: Control Group (P-0): Rat pellet feed + distilled water/day, Treatment Group I (P-1): Rat pellet feed + distilled water + Grape seed extract at 50 mg/kg BW, Treatment Group II (P-2): Feed rat pellets + distilled water + Grape seed extract at 100 mg/kg BW, Treatment Group III (P-3): 150 mg/kg BW, and Treatment Group IV (P-4): 200 mg/kg BW.

**Table 2. Normality Test Results**

| Groups | | Kolmogorov-Smirnov[a] | | | Shapiro-Wilk | | |
|---|---|---|---|---|---|---|---|
| | | Statistic | df | Sig. | Statistic | df | Sig. |
| Result | Control (P-0) | .201 | 5 | .200* | .881 | 5 | .314 |
| | Treatment I (P-1) | .210 | 5 | .200* | .973 | 5 | .892 |
| | Treatment II (P-2) | .198 | 5 | .200* | .938 | 5 | .654 |
| | Treatment III (P-3) | .236 | 5 | .200* | .932 | 5 | .611 |
| | Treatment IV (P-4) | .203 | 5 | .200* | .940 | 5 | .669 |

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

**Table 5. LSD Post Hoc Test**
**Dependent Variable: LSD Results**

| (I) Groups | (J) Groups | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| Control (P-0) | Treatment I (P-1) | .21980* | .02348 | .000 | .1708 | .2688 |
| | Treatment II (P-2) | .37340* | .02348 | .000 | .3244 | .4224 |
| | Treatment III (P-3) | .41060* | .02348 | .000 | .3616 | .4596 |
| | Treatment IV (P-4) | .51660* | .02348 | .000 | .4676 | .5656 |
| Treatment I (P-1) | Control (P-0) | -.21980* | .02348 | .000 | -.2688 | -.1708 |
| | Treatment II (P-2) | .15360* | .02348 | .000 | .1046 | .2026 |
| | Treatment III (P-3) | .19080* | .02348 | .000 | .1418 | .2398 |
| | Treatment IV (P-4) | .29680* | .02348 | .000 | .2478 | .3458 |
| Treatment II (P-2) | Control (P-0) | -.37340* | .02348 | .000 | -.4224 | -.3244 |
| | Treatment I (P-1) | -.15360* | .02348 | .000 | -.2026 | -.1046 |
| | Treatment III (P-3) | .03720 | .02348 | .029 | -.0118 | .0862 |
| | Treatment IV (P-4) | .14320* | .02348 | .000 | .0942 | .1922 |
| Treatment III (P-3) | Control (P-0) | -.41060* | .02348 | .000 | -.4596 | -.3616 |
| | Treatment I (P-1) | -.19080* | .02348 | .000 | -.2398 | -.1418 |
| | Treatment II (P-2) | -.03720 | .02348 | .029 | -.0862 | .0118 |
| | Treatment IV (P-4) | .10600* | .02348 | .000 | .0570 | .1550 |
| Treatment IV (P-4) | Control (P-0) | -.51660* | .02348 | .000 | -.5656 | -.4676 |
| | Treatment I (P-1) | -.29680* | .02348 | .000 | -.3458 | -.2478 |
| | Treatment II (P-2) | -.14320* | .02348 | .000 | -.1922 | -.0942 |
| | Treatment III (P-3) | -.10600* | .02348 | .000 | -.1550 | -.0570 |

*. The mean difference is significant at the 0.05 level.

**Note:** Control Group (P-0): Rat pellet feed + distilled water/day, Treatment Group I (P-1): Rat pellet feed + distilled water + Grape seed extract at 50 mg/kg BW, Treatment Group II (P-2): Feed rat pellets + distilled water + Grape seed extract at 100 mg/kg BW, Treatment Group III (P-3): 150 mg/kg BW, and Treatment Group IV (P-4): 200 mg/kg BW.

Table 1 also shows that all groups demonstrate wound healing in the Wistar white rat strain. The two groups' average percentages of healing were different. On the fourteenth day, the average wound healing percentage for groups P0, P1, P2, and P3 was 72.3%, 84.3%, 94%, and 93.9%, respectively. Plus, all of group P4. The results show that group P4 heals faster than P0, P1, P2, and P3.

The normalcy test using Shapiro-Wilk

was applied due to the small sample size (less than 50) [30]. Table 2 displays the outcomes of the One-Sample Kolmogorov-Smirnov test, which was used to determine if the data is normally distributed. Each group's results were statistically significant at the 0.200 level. Furthermore, findings greater than 0.05 are shown using the Shapiro-Wilk significance test. If the p-value exceeds 0.05, we say the data follows a normal distribution. According to this analysis, the data follows a normal distribution.

**Table 3. Homogeneity Test Results**

| | *Levene statistic* | df1 | df2 | Sig |
|---|---|---|---|---|
| Result | 3.734 | 4 | 20 | .200 |

We checked for treatment group homogeneity using the Levene test at a 5% significance level. According to Table 3, which shows the results of the Levene test for homogeneity, the significance column has a probability value of 0.200. Groups P0, P1, P2, and P3 are either wholly homogeneous or originate from populations with a variance equal to or more than 0.05 (p>0.05), the significance level.

**Table 4. One-Way ANOVA Test Results**

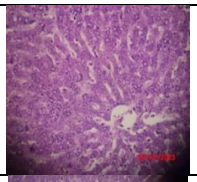
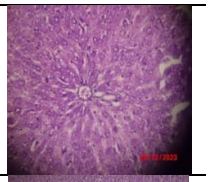
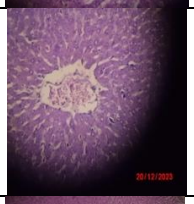
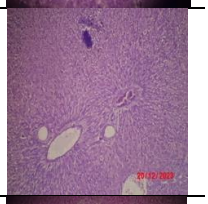
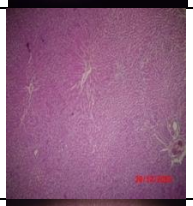
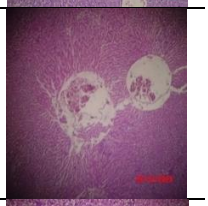
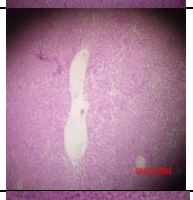
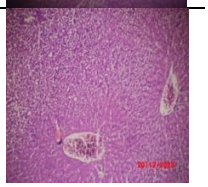
| Groups | Sum of Squares | df | Mean square | F | Sig |
|---|---|---|---|---|---|
| Between | .804 | 4 | .201 | 145.85 | .000 |
| In | .028 | 20 | .001 | | |
| Total | .832 | 24 | | | |

The significant value obtained from the One-Way ANOVA test is 0.000, less than 0.05, as shown in Table 4. From these numbers, we can deduce that the treatment groups in this study differed significantly. Next, we can examine the variations in average LDL values between the groups using the Post-hoc LSD further test.

Table 5 shows the results of the LSD Post Hoc Test, which is used to find out if there are any significant differences between groups. A significance value of 0.000, which is less than 0.05, indicates that the group has substantial differences from other groups, according to the results of the Post Hoc LSD test analysis in this study.

The goal of this observation was to examine the structure and morphology of cells, especially fibroblast cells, in each cut wound specimen in the treatment group with base cream (0), celery leaf extract cream at 50mg/kgBW, 100mg/kgBW, 150mg/kgBB, and 200mg/kgBB. Cut wounds heal by inflammation, proliferation, and maturation. Fibroblasts produce collagen and other wound-healing proteins during proliferation. Histopathological analysis of healed cut wounds with a 400x light microscope showed differences in fibroblast numbers in Table 6.

**Table 6. Histopathological Results**

| Groups | Histopathological Image of Fibroblasts | |
|---|---|---|
| | Before | After |
| Control (P0) |  |  |
| Treatment I (P-1) |  |  |
| Treatment II (P-2) |  |  |
| Treatment III (P-3) |  |  |
| Treatment IV (P-4) |  |  |

**Note:** Control Group (P-0): Rat pellet feed + distilled water/day, Treatment Group I (P-1): Rat pellet feed + distilled water + Grape seed extract at 50 mg/kg BW, Treatment Group II (P-2): Feed rat pellets + distilled water + Grape seed extract at 100 mg/kg BW, Treatment Group III (P-3): 150 mg/kg BW, and Treatment Group IV (P-4): 200 mg/kg BW.

## DISCUSSION

This study aimed to test the research hypothesis on the effect of grape seed extract (*Vitis vinifera l*) in the healing process of cuts in white rats (*Rattus norvegicus*) of the Wistar strain.

It is important to remember that cuts

and other open wounds are prime targets for bacteria and other microorganisms and can serve as entry points for systemic infections; hence, wound care is necessary [31]. Wounds in people with diabetes have an increased risk of not healing and becoming infected, as well as the potential for amputation [14].

Plants are hardwired from birth to carry out various physiological tasks and defend themselves against herbivores and microbes by producing diverse phytochemicals. The synthesis of these phytochemicals demonstrates plants' innate ability to react to stressful environmental situations [4]. This study determines whether grape seed cream preparations accelerate the healing process of cuts on the skin surface of white Wistar rats as test animal samples. Open wounds like cuts are susceptible to infection, especially by bacteria, and can lead to systemic diseases requiring wound care [31-32]. Plants contain flavonoids, saponins, and tannins that cure wounds. Hence, they are often used for wound care. Grape seed may cure wounds. Grape seed flavonoids are anti-allergic, anti-inflammatory, antiviral, anti-carcinogenic, and antioxidants that inhibit free radicals [32-33]. Flavonoids, saponins, and tannins are antioxidants, proangiogenesis, and oxygen-rich for wounded skin [23,35]. Thus, researchers want to know if grape seed extract cream speeds up the healing of white Wistar rat skin wounds.

Data from treatment procedures was collected for the study. The collected data is processed to test its normalcy. The researcher tested normalcy first. The data normality test showed a significance value of 0.200 > 0.05, indicating that the data was normally distributed and typical of the population. Homogeneity testing was then performed to determine subject variance. The significance level is 0.200. As the significance probability value is more significant than 0.05, all treatment groups come from a population with the same variance. Finally, a one-way ANOVA test determined significance. If the significance value is 0.000 or less than 0.05, H0 is rejected, or wound healing differs between groups, requiring a Post Hoc LSD follow-up test. The Post Hoc LSD test analysis in this study indicated a significance value of 0.000 or less than 0.05, indicating significant differences between groups.

A histopathological study of treated skin fibroblasts was also done. The P4 treatment group, which received 200mg/kg BW grape seed extract, had more and denser fibroblast cells than the P0 group, which received base cream without extract, P1, which received 50mg/kg BW, P2, which received 100mg/kg BW, P3, which received 150mg/kg BW, and P4. These findings showed that P4 wound recovery was better than the other groups.

Grape seed extract promoted wound healing. P4 at 10% accelerated wounds better than the other groups. This is shown by the fastest average healing acceleration and the only 100% healing group. Grape seed leaf extract contains flavonoids, saponins, and tannins that can prevent free radicals and be anti-allergic, anti-inflammatory, antiviral, anti-carcinogenic, and antioxidants.

Because the active catechin component in grape seeds aids the performance of the superoxide dismutase (SOD) enzyme, which serves to eliminate free radicals, this study is consistent with previous ones that discovered that grape seed extract could aid in the healing of wounds in rat liver tissue [35]. Another study found that hydroalcoholic grape seed extract helped rabbits recover from wounds. In this study [33], they discovered that GSE administration improved cutaneous wound healing in rabbits by increasing the oxidant activities of catalase (CAT) and glutathione peroxidase (GSH-Px) [34].

## CONCLUSION

This study found that grape seed extract cream (*Vitis vinifera L*) speeds wound healing in Wistar white rats. This is seen by the average wound healing on day 14 for groups P0 72.3%, P1 84.3%, P2 94%, and P3 93.9%. Group P4 100%. Thus, group P4 heals faster than P0, P1, P2, and P3. In the wound healing phase, the P4 treatment group given grape seed extract (Vitis vinifera L) at 200mg/kgBB had more fibroblast cells and denser cells than the other groups.

The study found that grape seed extract cream (*Vitis vinifera L*) increased skin regeneration and fibroblasts more than base cream. The grape seed extract (*Vitis vinifera L*) group closed incision wounds better than the base cream group. The grape seed extract group produced more wound-healing fibroblasts than the base cream group. Grape seeds include flavonoids, which are anti-inflammatory and antioxidants that prevent free radicals and speed wound healing.

According to phytochemical testing,

grape seed extract contains flavonoids, saponins, tannins, steroids, and alkaloids. These chemicals accelerate Wistar white rat back wound healing.

Further research is needed to determine if grape seed extract cream (*Vitis vinifera L*) improves human wound healing. Histopathological skin tissue studies to measure collagen and fibroblasts in people are also essential. Further human subjects study is required to determine the impact of grape seeds on wound healing. A comprehensive evaluation of the health-promoting compounds found in grape seeds, including vitamins, calcium, and potassium.

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